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SOIL BIOLOGICAL CHANGES OCCURRING AS A RESULT OF INTERMITTENT TILLAGE OF LONG-TERM NO-TILL SOILS

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Project Name: Soil Biological Changes Occurring as a Result of Intermittent Tillage of Long-Term, No-Till Soils

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Abstract/Summary:

Long-term, no-till and minimum-till management have become dominant production systems on the Canadian prairies. Despite the advantages in reducing soil erosion and improving soil quality, questions are arising about the economic viability of the system because of the heavy reliance on chemicals. Furthermore, many perennial weeds are difficult to control with herbicides and are increasingly problematic in mature no-till and minimum-till fields. This study investigated the impact of periodic tillage of long-term no-till soils on soil biological indices of soil quality Four treatments with different tillage intensities were investigated at three sites managed as no-till for a minimum of 10 years. Tillage had little long-term affect on the soil biological parameters at any of the sites. Only the most intensive black tillage treatment stimulated microbial populations and nutrient mineralization rates at Central Butte. Because this site had low inherent organic matter contents, this stimulation of activity could potentially deplete organic carbon levels. However, the effect was short-lived and by mid-season, there were no differences among the tillage treatments. None-the-less, the least intensive tillage to accomplish the management objective is recommended. At the Rosthern site black tillage stimulated parameters related to inorganic N supply, immediately after the implementation of the tillage. This improved N-supply in the black tillage treatment improved grain yields to levels of the no-till controls. The moderate tillage treatments reduced yields. There is little risk of depleting the soil organic matter supply by periodically tilling these soils because of the inherently higher organic matter contents compared to Central Butte. Soils at the Tisdale site exhibited the most extreme nutrient stratification in the top 10 cm. In general, soils at this site benefited from the implementation of the black tillage treatment, compared to the more moderate tillage treatments. All of the tillage treatments depressed the soil microbiological measurements compared to the no-till controls and the degree of depression was associated with the intensity of the tillage. However, several of the indices decreased from no-till to minimum till to conventional till, but then increased in the black tillage treatment back to near the levels of the no-till treatment. The more intensive tillage treatment was required for adequate soil mixing which benefited productivity.

Introduction:

Over the last several decades, long-term, no-tillage ('no-till') and minimal tillage farming systems have become dominant production systems on the Canadian prairies. Reducing the number and intensity of tillage operations minimizes soil loss through wind and water erosion, and stops or reduces the decline in soil organic matter (Campbell et al., 1996; Larney et al., 1997). The elimination of tillage and fallow has resulted in average increases in soil organic C of 200 kg C ha^{-1} yr^{-1} in the Brown soil zone and 400 kg C ha^{-1} yr^{-1} in the Dark Brown and Black soil zones (Liang et al., 1999). No-till soils had better soil structure and improved water infiltration (Carter et al., 1994) compared to conventional systems that led to reduced water erosion. In recent years, no-till systems have been promoted for their carbon sequestration abilities. No-till systems have reported soil organic carbon increases up to 1.5 Mg ha^{-1}. The overall improvement in soil structure and quality has led to increased net returns compared to systems incorporating intensive tillage (Larney et al., 1997; Singh and Singh, 1994).

Despite the wide reaching advantages obtained from reducing tillage, no-till has some potential drawbacks. No-till is essentially a mulch cropping system that relies heavily on herbicides for weed control without disturbing residues on the soil surface (Jones et al., 1990). Some producers implementing no-till management are starting to question the economic viability of this system in view of the increasing costs of fertilizers and herbicides, and low commodity prices (Larry Gutek, personal communication). Furthermore perennial weeds such as quackgrass, dandelion and foxtail barley are difficult to control with herbicides and are increasingly problematic in mature no-till and minimum-till fields (Derksen et al., 1993). Stratification of nutrients near the soil surface (Crozier et al., 1999) and slow spring soil warming because of heavy residue layers (Jones et al., 1990) present additional concerns

Some producers report that periodic tillage (every 7-10 years) may be necessary to control weeds and manage crop residues. However little is know about how these intermittent tillage events affect soil structure and quality. A study (ADF # 2003112-000274) conducted on the same plots used in the current study examined the effect of

tillage of long-term no-till plots on chemical and physical properties of the soil as well as crop productivity.

Soil quality and productivity are integrally linked to the soil biota. Soil organisms control the extent and rate of decomposition, control nutrient availability, provide the "glue" for soil aggregate formation, and contribute to the aeration and water-holding capacity of the soil. Conventional tillage of long-term no-till soils should stimulate overall microbial activity through enhanced aeration and soil warming. Spring surface soil temperatures can be up to 7 °C warmer under conventional tillage compared to no-tillage. In contrast, certain microbial populations, like denitrifiers, that require low oxygen conditions should be decreased. Generally speaking higher microbial activity together with incorporation of surface residues, should increase residue decomposition, possibly increasing levels of plant available nutrients like N and P. However, more active microorganisms may also mean more microbial immobilization that will lead to lower available amounts of these nutrients. It is often assumed that microbial stimulation means healthier, more productive soils. High levels of microbial biomass were associated with high levels of potentially available N (Hart et al., 1986; Stockdale and Rees, 1994) but the opposite was true for plant-available phosphorus and plant yield (Sorn-srivichai et al., 1988). Furthermore, soil respiration (a measure of total soil biological activity) was greater from less productive than more productive soils (Dinwoodie and Juma, 1988). In addition, stimulating decomposition could also result in rapid losses of the soil organic matter pool that accumulated because of the implementation of no-tillage.

Only about 10 % of microorganisms in soils are culturable in a laboratory, so it is impossible to get a complete understanding of how events like tillage or herbicide application impact the microbial communities in soils. Indices like microbial biomass, microbial biomass C and N can give some indication of how the entire community changes. Other indices like soil enzyme activities focus on the function that groups of organisms are performing. Ureases are an enzyme group that decompose urea to carbon dioxide and ammonium and are produced by many microbial sources as well as plants. Measurement of this enzyme activity gives an indication of the overall soil's ability to release ammonium from urea, and thus an indication of the dynamic N status in the soil.

Dehydrogenase catalyses the removal of hydrogen from organic compounds. The enzyme is unique in that it exists only in viable cells and thus is a general indicator of soil microbial activity. Alkaline phosphatase catalyses the hydrolysis of esters and anhydrides of H_3PO_4. It is a key enzyme in the mineralization of phosphorus from organic matter. Protease plays a key role in the release of inorganic N from organic molecules. It correlates well with microbial biomass estimates.

The objective of this research was to examine the influence of tillage, and tillage intensity, on the soil biology by investigating indices of biological activity. A secondary objective was to examine the persistence of any shifts in biological activity caused by tillage, ultimately to assess the degree of influence of tilling these 'no-till' soils.

Methods:

Field experiments:

Tillage treatments were applied in either the fall of 2004 or the spring of 2005. The no-till treatment had no pre-seeding treatment. The minimum tillage treatment was tilled with a single pass of a 1.8-m cultivator equipped with 30-cm sweeps and a 30-cm row spacing. Plots were tilled to an approximately 7- to 8-cm depth. The same cultivator was used for all treatments. The conventional tillage treatment included a tillage operation in the fall of 2004, followed by a spring tillage operation in 2005. The black tillage treatment, represented a very aggressive tillage operation and included the same operations as the conventional tillage treatments, followed by an additional tillage operation in the spring of 2005 using a tandem disc with notched blades.

Treatments were arranged in a completely randomized design replicated four times. The field sites were established by Dr. M. Grevers and were in place when the present study began. Soil sampling commenced in May 2005, immediately after the spring tillage operations were complete.

The Central Butte site is located in the Brown soil zone. It had been cropped in a cereal chem-fallow rotation using no-till management for 10 years. The Rosthern site is located in the Black soil zone on the Seager Wheeler farm. The site had been continuously cropped with a cereal-oilseed rotation for the last 12 years and was managed as a no-till site. The Tisdale site is located in the Gray soil zone. This site was

continuously cropped for the last 12 years with a cereal-oilseed rotation using no-till management. All of the sites met the criteria of being managed as no-till fields for a minimum of 10 years.

After the tillage treatments were applied, the Central Butte site was seeded to Hard Red Spring Wheat (*Triticum aestivum* L. cv Prodigy). Seeding rate was 94 kg ha^{-1}. A fertilizer blend (28-26-0) was seed placed at a rate of 67 kg ha^{-1}. The Rosthern site was seeded to canola (*Brassica napus* L. cv Banner) at a rate of 6.2 kg ha^{-1}. Blended fertilizer (56-22-11) was side-banded at seeding. The Tisdale site was seeded to canola (*B. napus* L. cv Millenium) at a rate of 5.6 kg ha^{-1}. Fertilizer (67-22-22) was applied at seeding. Half of the N fertilizer was placed mid-row, the remaining N-fertilizer was seed-placed with the P and S.

Soil sampling and laboratory analyses:

Soils were sampled at four to five week intervals throughout the growing season. The first samples were collected immediately after the spring tillage operations were complete. The last samples were collected in October prior to freeze up. Five samples were taken randomly from each plot and bulked to form one composite sample. Individual samples were removed with a soil probe with a 7-cm diameter to a 10-cm depth. After thoroughly mixing, the composite sample was subdivided into two sub-samples. One sub-sample was refrigerated (4°C) and extracted for nutrients. The second sub-sample was frozen (-20 °C) and used for the enzyme activity analyses. A preliminary experiment determined that the effects of the tillage treatments were only observable in the plough-layer and not deeper in the soil profile.

Although samples were collected each month, only a sub-sample of the total set was analysed. The purpose of the monthly sampling was to determine the length of time the tillage affect lasted. This was achieved with a subset of the total samples. Furthermore, the amount of time required to complete the enzyme assays prohibited the analysis of all of the samples.

Nitrification is the microbially mediated conversion of NH_4^+ into NO_2^- and ultimately NO_3^- in the presence of oxygen. Net nitrification is measured as the accumulation of NO_3^- during aerobic incubation (Drury et al., 2008a). Twenty g

subsamples of soil were placed in vials, water added to achieve field-capacity and the vial covered with parafilm. A small hole was punctured in the parafilm for air exchange and to enable water to be added, and at the same time minimize water lost from evaporation. Water was applied as needed by weighing a sub-sample of vials, and determining the average water lost from the vials. The average mass of water lost was added to all of the vials. Watering was only necessary an average of once a week, when 20 % of the water volume was lost. Vials were incubated in a controlled temperature cabinet at 20°C in the dark. Open pans of water were left in the chamber to maintain a high relative humidity in the chamber. Vials were never watered within 48 h of the extraction procedure, because adding water can cause a flush of nutrient release. Sampling was conducted at 0, 1, 2, 4, 6 and 8 wks. At each sampling period, four vials were selected randomly and extracted for NO_3^- using KCl as the extractant. Extracts were analysed on an autoanalyzer.

Net N-mineralization and net P-mineralization were measured using similar aerobic incubations (Curtin and Campbell, 2008). Soils were prepared and incubated in the same manner as the nitrification experiment. Vials were sampled after 0, 1, 2, 4, 6 and 8 days. At each of the sampling periods, 4 vials were extracted with KCl and total inorganic N determined. A second set of 4 vials was extracted with modified Kelowna extractant (Qian et al., 1994) and analysed for phosphate.

During denitrification NO_3^- is converted to N_2O and N_2 gas, mediated by soil microorganisms. The acetylene reduction method for estimating denitrification potential forces all of the NO_3^- to be converted to N_2O rather than the alternate N_2. Potential denitrification represents the total potential of the soil under the incubation conditions, to produce N_2O. Denitrification potential was measured according to the method outlined by Drury et al. (2008b). Subsamples of sieved (4mm), field-moist soil were placed in a sealed glass chamber fitted with a sampling port. Air was removed from the chamber using a syringe. An equal volume of acetylene was injected into the negative airspace. Soils were incubated at room temperature in the dark for 24 h. Subsamples of the airspace were removed from each jar and analysed by gas chromatography for N_2O production.

The enzyme assays included in this study were selected to provide insight into ecosystem functions, specifically as indicators of overall microbial activity and their role

in mineralization of major nutrients. Soil enzymes act as the mediators and catalysts of important soil functions like decomposition of organic matter, mobilization of plant nutrients, and nitrification (Dick, 1977).

Dehydrogenase activity was measured using the TTC method that uses 2,3,5-triphenyltetrazolium chloride (TTC) as an NAD^+ inhibitor. Exclusion of oxygen favors the transfer of electrons to TTC, reducing the TTC to a red triphenyl formazan (TPF) (Pepper and Gerba, 2004). The method was performed according to (Casida, 1977) with modifications. Six grams of soil was placed in a glass vial with 100 *M*m TRIS buffer (pH 7.6) and 6 mL of TTC solution. The samples were incubated for 24 h at room temperature, filtered and rinsed with methanol. Optical density was measured at 485nm with the Thermospectronic Spectronic 20D+ (Thermo Fischer Scientific Inc., Waltham, MA)

Alkaline phosphatase activity was measured according to the method described by (Tabatabai and Bremmer, 1969). This method uses a phosphorus (p-nitrophenyl phosphate) substrate mixed with the soil to promote hydrolysis and release of orthophosphate. The yellow coloration of the solution is analysed at 420nm using the Thermospectronic Spectronic 20D+.

Protease activity was determined using the method of Ladd and Butler (1972) where casein was added as a substrate and the subsequent release of amino acids was measured. One g of wet soil was mixed with 5 mL of 50*M*m TRIS buffer (pH 8.1) and 5 mL of 2% sodium caseinate solution. The tubes were incubated at 50°C for 2 h. The reaction was stopped with 15% trichloroacetic acid solution (TCA). The suspension was centrifuged at 10,000 rev min^{-1} for 10 min. Five mL of supernatant was mixed with 7.5 mL alkaline reagent and incubated for 15 min at room temperature. Five mL of 33% Folin-Ciocalteu was added. After the solution was filtered the absorbance was measured after exactly 1 h at 700 nm with the Thermospectronic Spectronic 20D+.

Urease activity was measured using methods described by Kandeler and Gerber, (1988) where the urease activity was determined by the amount of NH_4^+ that is released when a 5 g sample of soil is incubated at 37°C for 2 h with 2.5 mL of urea solution (2.4 g urea dissolved in 500 mL water). After the incubation the sample was extracted with a 50 mL KCl solution shaken for 30 min and filtered . The amount of ammonium was

determined on 1 mL filtrate mixed with 9 mL distilled water, 5 mL sodium salicylate/NaOH(0.1*M*) solution and 2 mL 0.1% sodium dichloroisocyanide solution. The mixture was incubated at room temperature for 30 min and the optical density measured at 690 nm using the Thermospectronic Spectronic 20D+.

Microbial biomass N and C were measured by the microwave (MW) irradiation method (Islam and Weil, 1998) with modifications. The soils were sieved through a 2 mm screen and brought to 80% water filled pore space (WFP). The energy output of the microwave was measured according to Neas and Collins (1988). Duplicate 25 g oven dried equivalent (ODE) sub-samples of soil were incubated at room temperature in plastic aerated vials. After 24 h incubation one of the subsamples was exposed to 2 x 17 s bursts of 400 J g^{-1}. The second sub sample was retained as a control. Both samples were then extracted with 100 ml 0.5 M K_2SO_4, shaken for 1 h at 150 rpm and filtered. TOC and N were measured using standard techniques (Carter and Gregorich, 2008). Microbial biomass N and C are calculated as the difference between the control sample and the micro waved sample. This difference represents the flush of nutrients released from the dead microbial cells, killed by microwave exposure.

Statistical analyses:

Statistical analyses were performed using SPSS ver. 16.0. Data were analysed as a completely randomized design with four replicates. Multiple comparisons were performed at α=0.10 using Fisher protected least significant difference test (lsd). Only statistically significant differences are indicated on Tables and Figures.

Results:

Overall microbial activity:

Carbon dioxide evolution, microbial biomass and dehydrogenase activity are all measurements of overall microbial activity and hence the functional quality of the soil. Cumulative CO_2 evolution from the Central Butte soil was only minimally affected by the tillage treatments (Fig. 1). The most intensive tillage treatment (black tillage) stimulated CO_2 production after 4 wks of incubation in the May samples. By July and continuing into October there was no difference in CO_2 generated from the four tillage treatments.

Carbon dioxide evolution from the Rosthern and Tisdale sites was unpredictable in response to the tillage treatments (Figs. 2 & 3). At Rosthern, none of the treatments showed any difference in CO_2 evolution at the early sampling (Fig. 2). Mid-season, CO_2 evolution from the conventionally tilled soil was lower than the other treatments, and by October, the conventional and black tillage treatments were lower.

At Tisdale (Fig. 3) as the intensity of tillage increased, the cumulative CO_2 produced tended to be reduced at the May sampling. This pattern continued into July except that the black tillage treatment exceeded all of the other treatment in CO_2 production. Black tillage appears to stimulate microbial activity during crop growth, possibly by altering the microbial communities associated with the roots of growing crops. No differences were evident by October. Generally speaking, tillage is expected to stimulate microbial activity and hence CO_2 evolution because of the introduction of O_2 into the soil. Because soil structure in long-term no-tillage soils is improved compared to conventionally tilled soils, it appears that O_2 is not limiting microbial activity in these no-till plots. Instead of stimulating microbial activity, the soil drying that accompanies tillage may be responsible for the moderate depression of CO_2 production experienced at the Tisdale site early in the season. Soils at the Tisdale site contained the most C and N compared to the other sites (Table 1) and hence the most organic matter for degradation. This is reflected in the overall higher CO_2 emissions from the Tisdale soil.

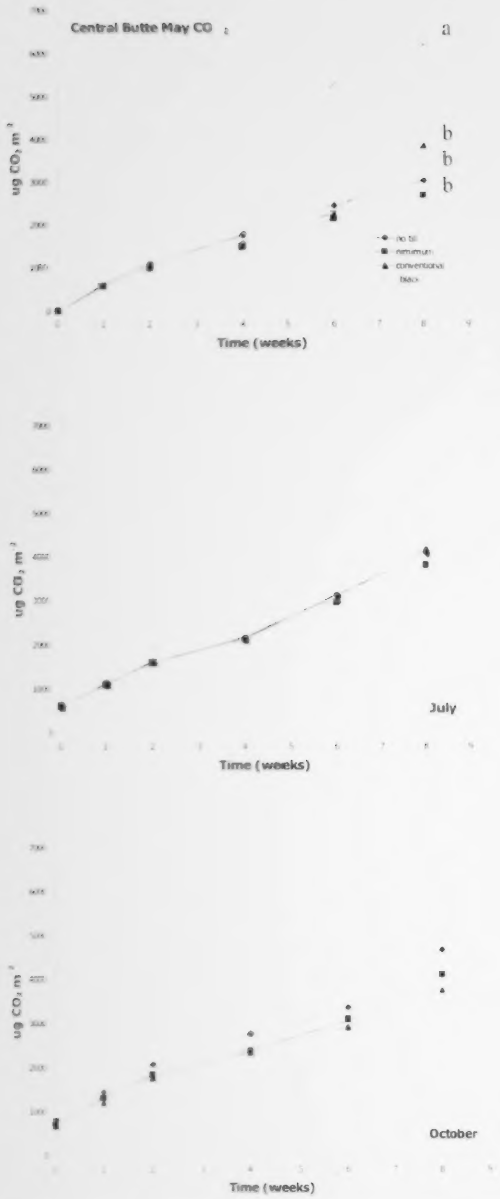


Figure 1: Cumulative carbon dioxide production from Central Butte long-term no-tillage plots receiving different intensities of a one-time intermittent tillage operation. May soil samples were collected immediately after the plots were tilled; July samples were collected two months after the tillage operations and October samples collected four months after the tillage operations. Different letters indicate statistical differences ($lsd_{0.10}$).

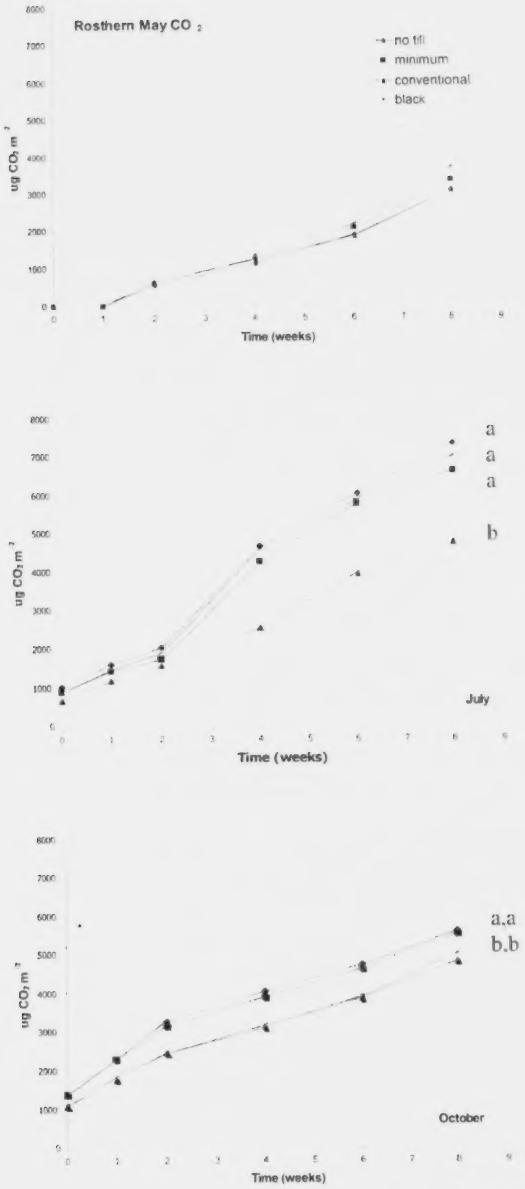


Figure 2: Cumulative carbon dioxide production from Rosthern long-term no-tillage plots receiving different intensities of a one-time intermittent tillage operation. May soil samples were collected immediately after the plots were tilled; July samples were collected two months after the tillage operations and October samples collected four months after the tillage operations. Different letters indicate statistical differences ($lsd_{0.10}$).

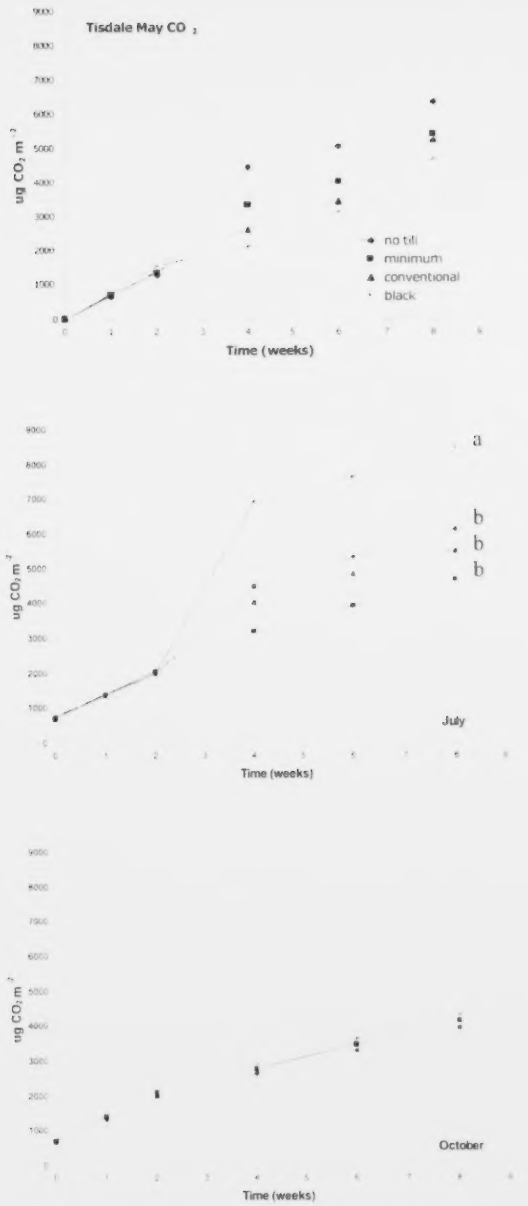


Figure 3: Cumulative carbon dioxide production from Tisdale long-term no-tillage plots receiving different intensities of a one-time intermittent tillage operation. May soil samples were collected immediately after the plots were tilled; July samples were collected two months after the tillage operations and October samples collected four months after the tillage operations. Different letters indicate statistical differences ($lsd_{0.10}$).

Table 1. Mean values of soil characteristics (0-15 cm) prior to tillage treatments being implemented at the study sites.

Site	pH	Bulk Density ($g\ cm^{-3}$)	Moisture[1] (%)	C_{tot} (%)	N_{tot} (%)	C:N
C. Butte	7.4	1.51	19.0	1.1	0.10	11
Rosthern	6.4	1.27	18.5	2.1	0.16	13
Tisdale	6.5	1.20	22.5	4.5	0.24	19

[1] 0-30 cm depth

Stratification of nutrients near the soil surface and slow spring soil warming because of heavy residue layers were more pronounced in the no-till treatment at the Tisdale site than the Rosthern or Central Butte sites (Baan, 2007). Presumably the shorter growing season and cooler overall temperatures experienced in the northern regions of the province where Tisdale is located accounted for the slower breakdown of organic matter and thicker organic layers at the soil surface. The mixing of soil at Tisdale from the intensive black tillage treatment might have stimulated microbial activity accounting for the higher CO_2 emissions.

Microbial biomass C and N were affected differently by tillage depending on the time of sampling and the site sampled. As was the case with cumulative CO_2 emissions (Fig. 1), both microbial biomass C (Fig. 4 top) and N (Fig. 5 top) were stimulated in the black tillage treatment at the May sampling of Central Butte compared to the other treatments. In October, microbial biomass N was very low at Central Butte, indicating that N immobilization at this site was very low late in the season. This is expected as the input of fresh organic matter from harvest had just occurred, and had not been actively broken down by the decomposer communities yet. Microbial biomass C increased from May to October at Central Butte. There was a tendency for microbial biomass C to increase with tillage intensity, except in the most intensively tilled black tillage treatment. Organic C levels are very low at this site (Table 1) and it may be that the readily available substrate for the microbial communities was depleted late in the season.

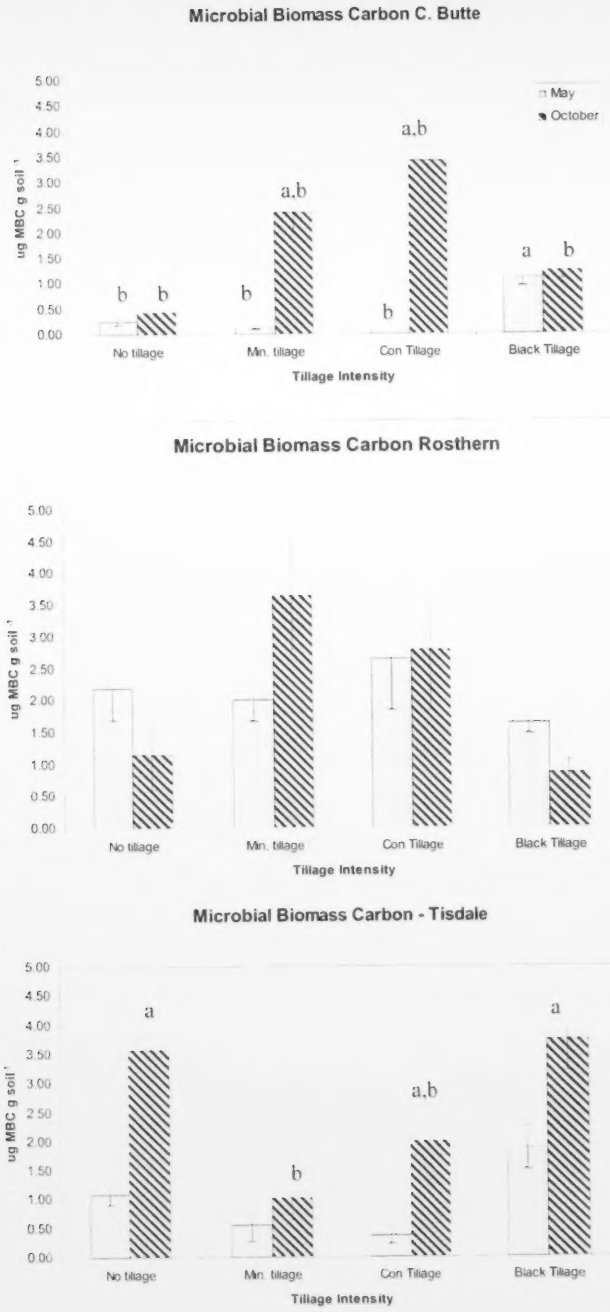


Figure 4. Microbial biomass carbon at three long-term no-tillage sites tilled once with different intensities of tillage. Values (se) are mean concentrations in soils immediately after tillage (May) and post-harvest of the same year (October). Different letters indicate statistical differences ($lsd_{0.10}$) within a sampling time.

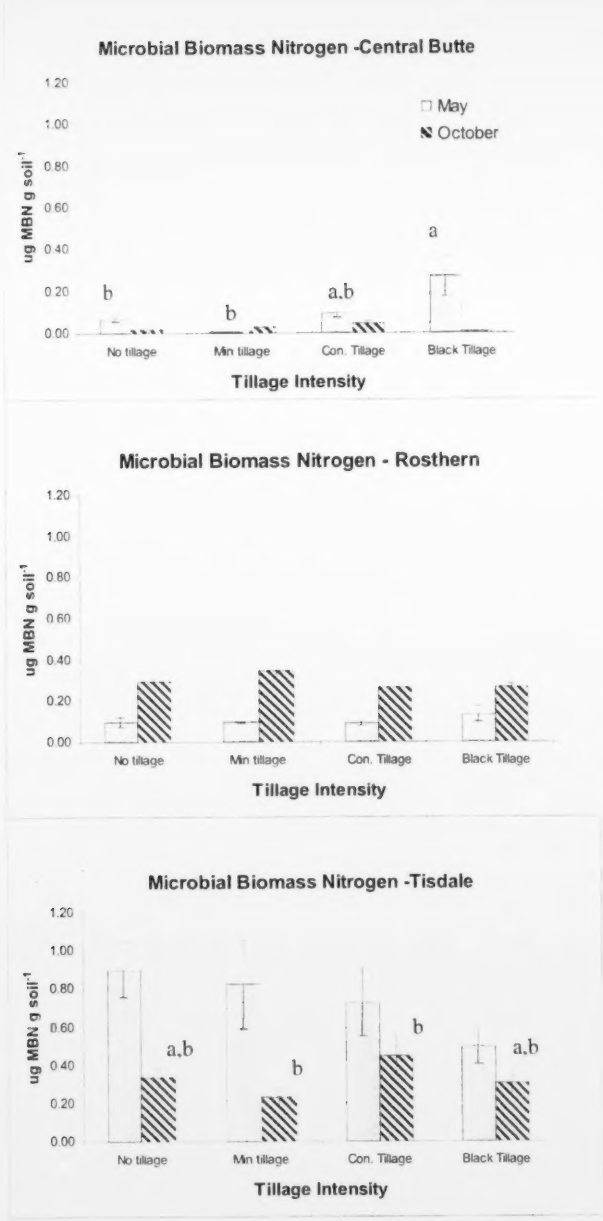


Figure 5. Microbial biomass nitrogen at three long-term no-tillage sites tilled once with different intensities of tillage. Values (standard error) are mean concentrations in soils immediately after tillage (May) and post-harvest of the same year (October). Different letters indicate statistical differences (lsd $_{0.10}$) within a sampling time.

Microbial biomass C and N at Rosthern (Figs. 4 and 5 middle) were generally unaffected by tillage treatment at either sampling time. The variability in microbial biomass C at this site was very large. The black tillage treatments tended to have reduced microbial biomass C numbers at Rosthern but the differences were not statistically different.

At Tisdale, intensive black tillage increased microbial biomass C compared to the other treatments, but reduced microbial biomass N at the early sampling (Figs. 4 & 5 bottom). This is indicative of a shift in C:N ratios of the microbial biomass, where N was more limiting in the more intensive tillage treatments. C:N ratios less than 20 indicate that a soil is conducive to net mineralization occurring. All of the sites had C:N under 20 (Table 1), but the value of 19 at Tisdale indicates that it is close to the transition between mineralization and immobilization. By October, the microbial biomass C levels were higher than they were in May, whereas the microbial biomass N levels were lower. This reflects that mineralization and uptake of N occurred over the growing season with a shift to immobilization late in the season. In October the no-till and black tillage treatments had the same microbial biomass C levels, but both were higher than the two moderate tillage treatments. As was the case with CO_2 production, it appears that at this site, where nutrients and organic matter are strongly stratified, the intensive tillage is the only operation that effectively mixes the soil strata thereby stimulating microbial activity.

Dehydrogenase activity is a general measure of microbial activity. Unlike many enzymes it is unique in that it is produced only in viable, microbial cells. Other enzymes can be released from dead, excised cells and still retain their function. Some of these exogenous enzymes can be released from plant cells as well as microbial cells. While all sources of the enzymes are ultimately important in the overall functioning of the soil, measurement of dehydrogenase activity reflects only the viable microbial population. Dehydrogenase catalyses the release of ionically bound water from organic matter during its decomposition and thus is a general indicator of degradation.

In the spring after tillage was performed there was a slight reduction in dehydrogenase activity consistently among the minimum and conventional tillage treatments at all of the sites (Fig. 6).

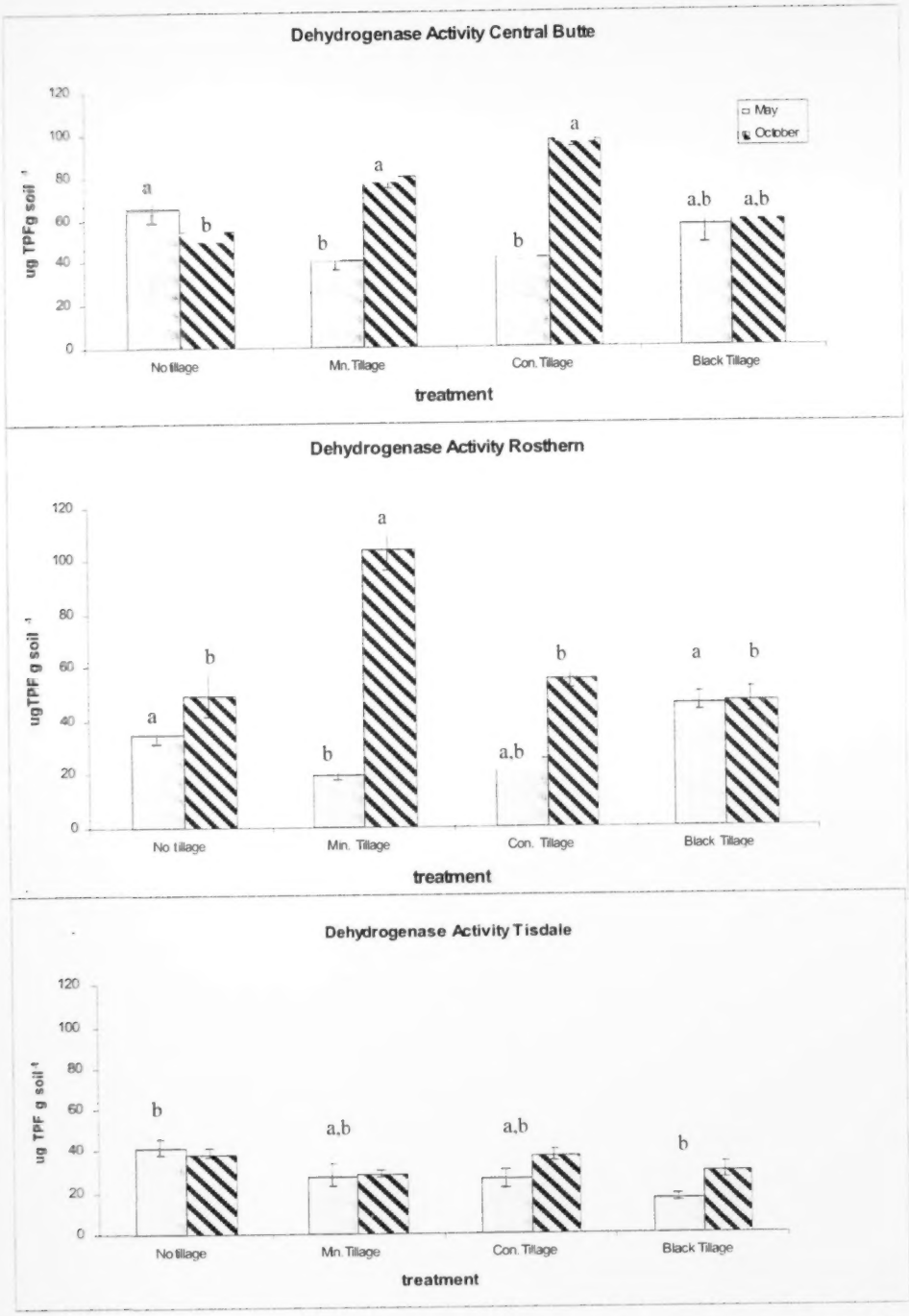


Figure 6. Dehydrogenase activity at three long-term no-tillage sites tilled once with different intensities of tillage. Values (standard error) are enzyme activities in soils immediately after tillage (May) and post-harvest of the same year (October). Different letters indicate differences (lsd $_{0.10}$) within a sampling time.

There was no difference between the minimum and conventional tillage treatments, but they both had lower dehydrogenase activity compared to the no-till treatment. Black tillage affected dehydrogenase activity differently at all of the sites. At Central Butte, dehydrogenase activity at the May sampling of the black tillage treatment was not different from any of the other tillage treatments (Fig 6 top). At Rosthern black tillage enhanced dehydrogenase activity compared to the two moderate tillage treatments bringing it to a comparable level to the untilled soil (Fig. 6 middle). At Tisdale black tillage reduced dehydrogenase activity relative to all other treatments (Fig. 6 bottom).

October samples tended to show reverse trends to the May samples at Central Butte in that the minimum tillage and conventional tillage treatments had higher dehydrogenase activities than the no-till and black tillage treatments (Fig. 6 top). Only the minimum tillage treatment at Rosthern showed enhanced dehydrogenase activity (Fig. 6 middle) and there was no difference in dehydrogenase activity in October at the Tisdale site (Fig 6 bottom). Dehydrogenase activities were more stable at Tisdale than the other two sites, probably because of the larger supply of organic matter available at this site (Table 1). The modest differences in dehydrogenase activity at all of the sites indicates that tillage was not particularly effective in stimulating organic matter breakdown, in the short-term.

Biological controls of nitrogen availability:

Nitrification is the two-step conversion of ammonium (NH_4^+) into nitrite (NO_2^-) and then into nitrate (NO_3^-). The conversion is catalysed by two genera of bacteria (*Nitrosomonas* and *Nitrobacter*). Net nitrification measurements give an indication of the overall activity of nitrifying bacteria; one step in the N-mineralization process. Changes in soil O_2 status, substrate availability (NH_4^+) and soil temperature have the potential to affect nitrifying populations and rates of conversion. At all of the sites (Figs. 7, 8 & 9) the implementation of different intensities of tillage on the previously no-till sites had an effect on net nitrification immediately after the tillage treatment was implemented (May sampling). At Central Butte (Fig. 7) and Rosthern (Fig. 8) nitrification was enhanced in the black tillage treatment. The moderate tillage treatments (minimum and conventional) did not affect net nitrification compared to the no-till treatment at either site.

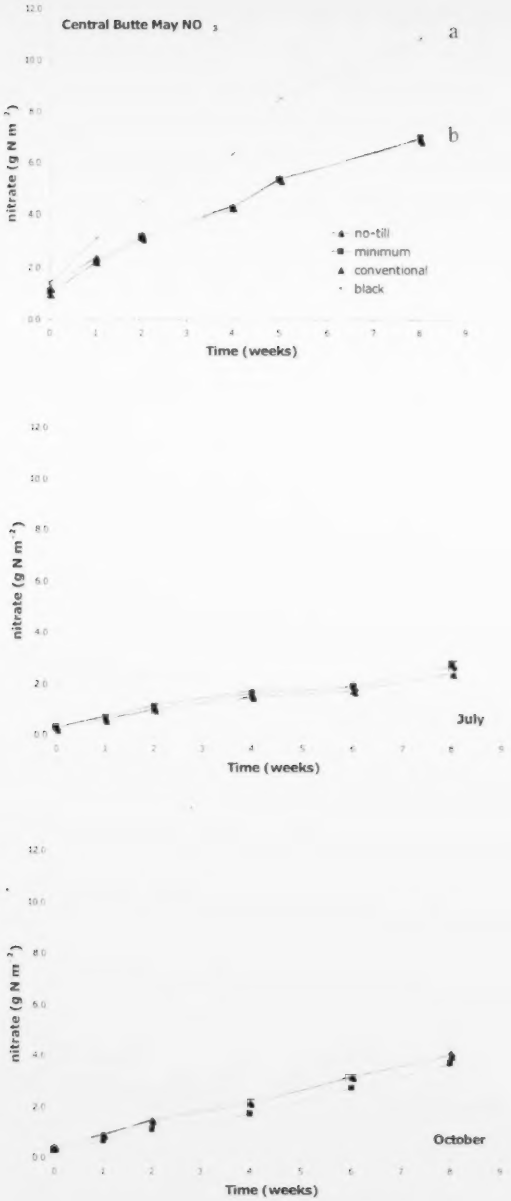


Figure 7: Cumulative nitrate production (net nitrification) from Central Butte long-term no-tillage plots receiving different intensities of a one-time intermittent tillage operation. May soil samples were collected immediately after the plots were tilled; July samples were collected two months after the tillage operations and October samples collected four months after the tillage operations. Different letters indicate statistical differences ($lsd_{0.10}$).

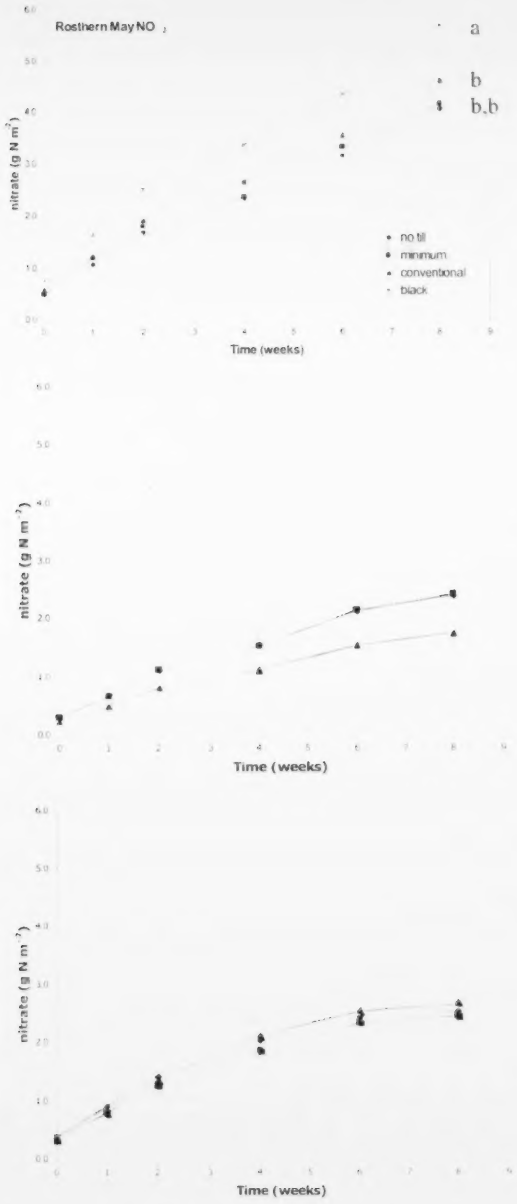


Figure 8: Cumulative nitrate production (net nitrification) from Rosthern long-term no-tillage plots receiving different intensities of a one-time intermittent tillage operation. May soil samples were collected immediately after the plots were tilled; July samples were collected two months after the tillage operations and October samples collected four months after the tillage operations. Different letters indicate statistical differences ($lsd_{0.10}$).

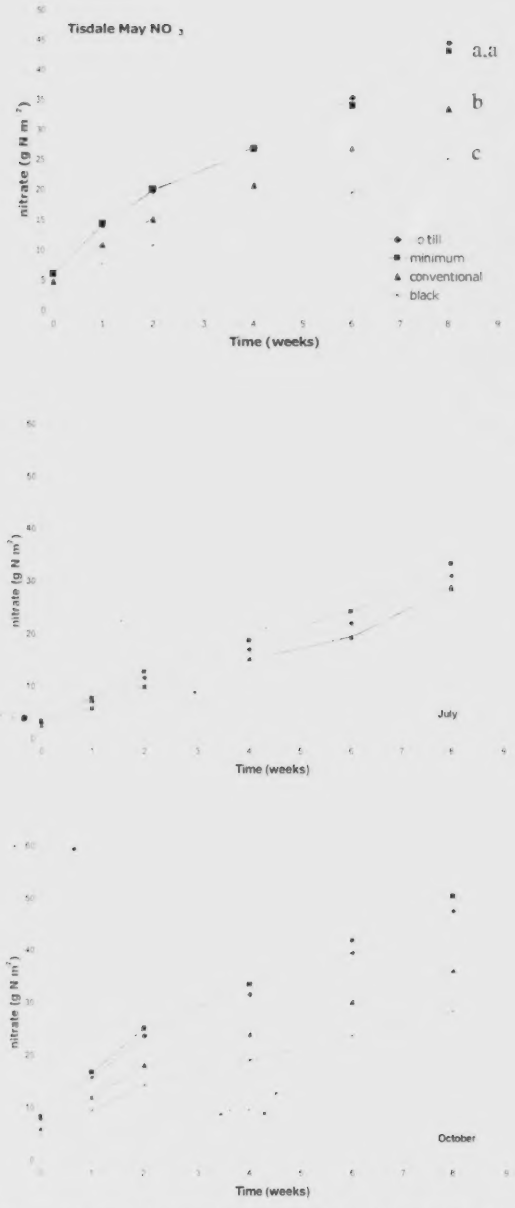


Figure 9: Cumulative nitrate production (net nitrification) from Tisdale long-term no-tillage plots receiving different intensities of a one-time intermittent tillage operation. May soil samples were collected immediately after the plots were tilled; July samples were collected two months after the tillage operations and October samples collected four months after the tillage operations. Different letters indicate statistical differences ($lsd_{0.10}$).

Enhancement of nitrification was probably the result of enhanced aeration, and mixing of organic matter throughout the plough layer exposing the substrate to a larger volume of soil with nitrifying bacteria. The less intensive tillage operations open the soil surface but do not cause complete mixing of the soil layers in the same manner that the black tillage operation did. By the mid-season sampling in July all of the treatments had stabilized at the Central Butte site and there were no differences among any of the tillage treatments in either July or October (Fig. 7). In contrast, differences in net nitrification among tillage treatments were apparent at the mid-season sampling at Rosthern (Fig. 8). Net nitrification was lower in the two most intensively tilled treatments possibly due to a greater depletion of substrate NH_4^+. By October differences among the tillage treatments no longer existed.

The Tisdale site responded very differently to the tillage treatments in terms of net nitrification, than the other two sites (Fig. 9). The two high intensity tillage treatments decreased net nitrification compared to the no-till and minimum tillage treatments and the difference was sustained throughout the season. Nitrification rates at the Tisdale site were much higher than at the other two sites, especially in May and October. Initial NO_3^- concentrations (Table 2) were approximately 10 x higher in May at Tisdale compared to Central Butte and Rosthern, and the no-till and minimum-tillage treatments had the highest NO_3^- concentrations. These higher substrate concentrations probably accounted for the higher nitrification rates at this site, and were reflected in the higher initial NH_4^+ concentrations (Table 2). As was expected, the July samples all had the lowest nitrification rates, regardless of tillage treatment. The actively growing crop was taking up NH_4^+ released into the soil and depleting the substrate for nitrification.

Net N mineralization reflects the overall conversion of organic N into inorganic N (NH_4^+ and NO_3^-). It is a measure of the total amount of inorganic N released for plant uptake. Organic N is released when microorganisms decompose organic matter into plant available inorganic forms. Some of the released N can be assimilated (immobilized) by microorganisms. Net mineralization represents the net production of inorganic N, after some immobilization has taken place. Environmental factors that affect mineralization are the same as those that affect nitrification, namely, substrate availability, O_2 and optimal temperature all favor mineralization.

Table 2: Nutrient concentrations at each sampling period at the three previously no-till sites that were tilled with different intensities of tillage. Values are means (standard deviation) of 4 replicates. There were no significant differences in nutrient concentrations among tillage treatments within a sampling date at any of the sites.

	Central Butte								
	May			July			October		
	NH_4	NO_3	PO_4	NH_4	NO_3	PO_4	NH_4	NO_3	PO_4
	($ug\ g^{-1}$)			($ug\ g^{-1}$)			($ug\ g^{-1}$)		
No-till	0.69	1.84	1.42	0.11	0.57	2.14	0.19	0.75	1.70
	(0.29)	(0.24)	(0.14)	(0.08)	(0.21)	(0.25)	(0.07)	(0.09)	(0.12)
Minimum	0.69	1.97	1.38	0.12	0.59	2.24	0.18	0.56	1.53
	(0.25)	(0.09)	(0.14)	(0.10)	(0.09)	(0.13)	(0.06)	(0.03)	(0.27)
Conventional	0.73	2.35	1.70	0.13	0.52	2.15	0.15	0.08	1.55
	(0.21)	(0.86)	(0.21)	(0.06)	(0.07)	(0.32)	(0.05)	(0.14)	(0.29)
Black	0.74	2.47	1.64	0.16	0.63	2.41	0.15	0.75	1.80
	(0.27)	(0.29)	(0.20)	(0.09)	(0.20)	(0.42)	(0.08)	(0.03)	(0.32)
	Rosthern								
No-till	0.10	1.08	2.23	1.16	0.68	2.54	0.22	0.78	1.82
	(0.05)	(0.26)	(0.31)	(0.25)	(0.06)	(0.44)	(0.03)	(0.11)	(0.29)
Minimum	0.23	1.09	2.10	1.02	0.69	2.35	0.20	0.68	1.53
	(0.17)	(0.15)	(0.39)	(0.32)	(0.20)	(0.39)	(0.04)	(0.18)	(0.29)
Conventional	0.15	1.27	1.93	1.10	0.56	1.90	0.23	0.87	1.38
	(0.06)	(0.21)	(0.33)	(0.33)	(0.10)	(0.31)	(0.08)	(0.12)	(0.28)
Black	0.12	1.69	1.89	1.06	0.53	2.02	0.24	0.87	1.43
	(0.05)	(0.24)	(0.28)	(0.36)	(0.06)	(0.16)	(0.11)	(0.16)	(0.16)
	Tisdale								
No-till	1.51	11.8	1.70	0.62	0.77	2.10	0.37	1.03	1.47
	(0.85)	(3.9)	(0.52)	(0.10)	(0.15)	(0.44)	(0.12)	(0.26)	(0.39)
Minimum	1.71	11.5	1.79	0.54	0.81	2.03	0.47	1.15	1.51
	(0.30)	(4.6)	(0.21)	(0.04)	(0.18)	(0.30)	(0.19)	(0.26)	(0.18)
Conventional	1.13	9.1	1.49	0.44	0.63	2.05	0.26	0.84	1.29
	(0.18)	(3.6)	(0.28)	(0.10)	(0.12)	(0.15)	(0.09)	(0.24)	(0.31)
Black	0.68	5.7	1.83	0.49	0.62	2.48	0.42	1.18	1.68
	(0.17)	(4.6)	(0.40)	(0.10)	(0.10)	(0.34)	(0.19)	(0.34)	(0.39)

However, organic matter (residue) quality also has a large effect on whether mineralization or immobilization is favored. Wide C:N (>30:1) ratios favor immobilization, whereas narrow ratios (<20:1) favor mineralization. Intermediate ratios (20 to 30) are thought to have a balance between mineralization and immobilization.

Patterns of net N mineralization (Figs. 10, 11, & 12) generally mirrored the patterns for net nitrification (Fig. 7, 8, & 9) at all three sites. N mineralization was only enhanced in the black tillage treatment at Central Butte and Rosthern early in the season (Figs. 10 & 11). There were no differences in net mineralization by July and continuing into October, indicating the short-lived nature of the enhancement. Patterns of net N mineralization were different at Tisdale than the other two sites. As was the case with net nitrification, N- mineralization rates were the highest at Tisdale compared to Central Butte and Rosthern; reflective of the higher organic matter content at this site (Table 1). Conventional tillage and black tillage depressed net N mineralization immediately after the treatments were imposed in May (Fig. 12). There was no difference among treatments mid-season when the crop was actively growing and taking-up N, but after crop removal in October, the rates were again lower in the two most intensively tilled treatments. In a soil where N mineralization rates are inherently low, due to low organic matter levels, this depression in mineralization activity could present a problem for crop growth, because of inadequate supply of N. However, in this Gray Tisdale soil, N mineralization rates are very high, and it is unlikely that the lower rates would limit N supply. It appears that stratification of nutrients and microorganisms is functionally more prevalent in the Tisdale soil than the Central Butte or Rosthern soils. The shorter growing season and cooler temperatures occurring in the Gray soil zone encourage a thicker thatch in the surface of these soils, encouraging microbial activity to concentrate in this region. Moderate to intensive tillage may cause a dilution of microbial populations responsible for controlling nutrient availability. Overall, in a situation where mineralization rates are inherently high, such as at Tisdale, the slower mineralization due to the implementation of tillage, should mean an extended window, long-term when N is available for crop uptake.

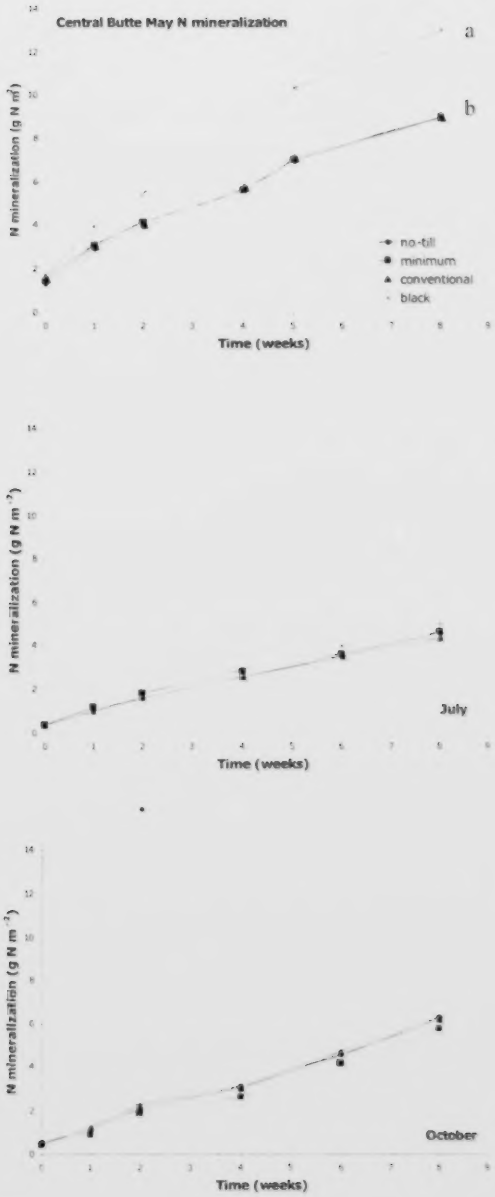


Figure 10: Net N mineralization from Central Butte long-term no-tillage plots receiving different intensities of a one-time intermittent tillage operation. May soil samples were collected immediately after the plots were tilled; July samples were collected two months after the tillage operations and October samples collected four months after the tillage operations. Different letters indicate statistical differences ($lsd_{0.10}$).

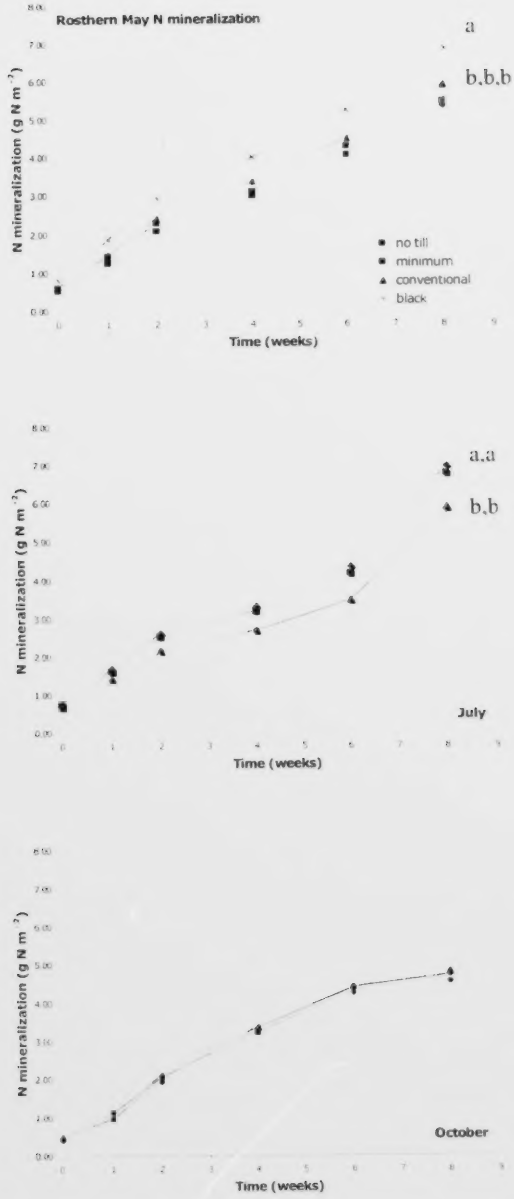


Figure 11: Net N mineralization from Rosthern long-term no-tillage plots receiving different intensities of a one-time intermitten tillage operation. May soil samples were collected immediately after the plots were tilled; July samples were collected two months after the tillage operations and October samples collected four months after the tillage operations. Different letters indicate statistical differences ($lsd_{0.10}$).

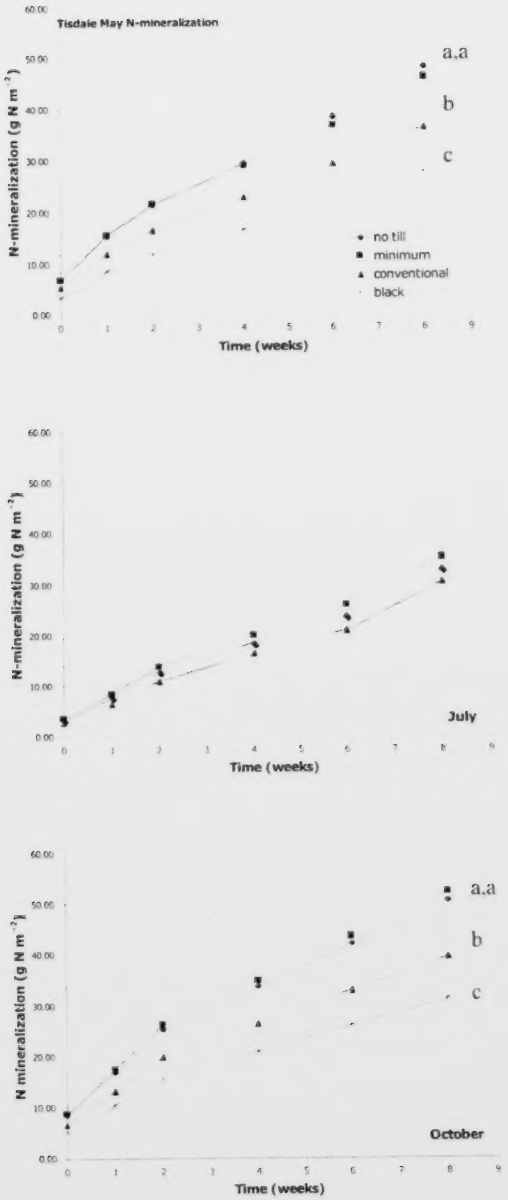
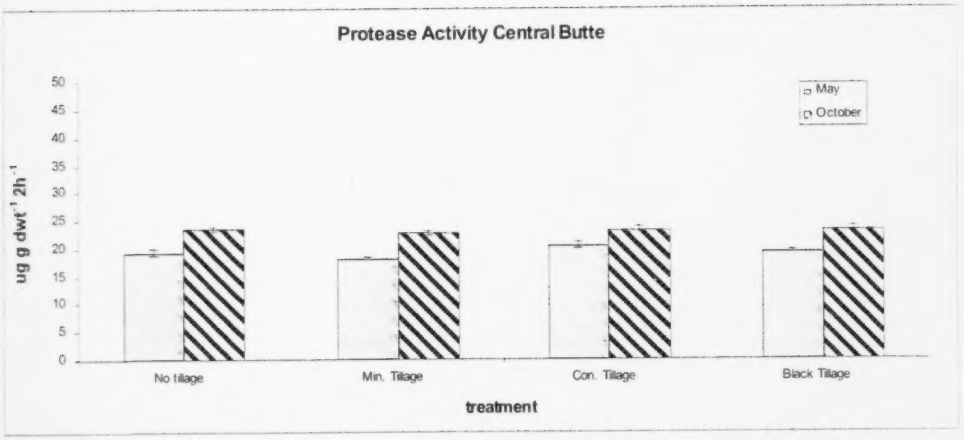
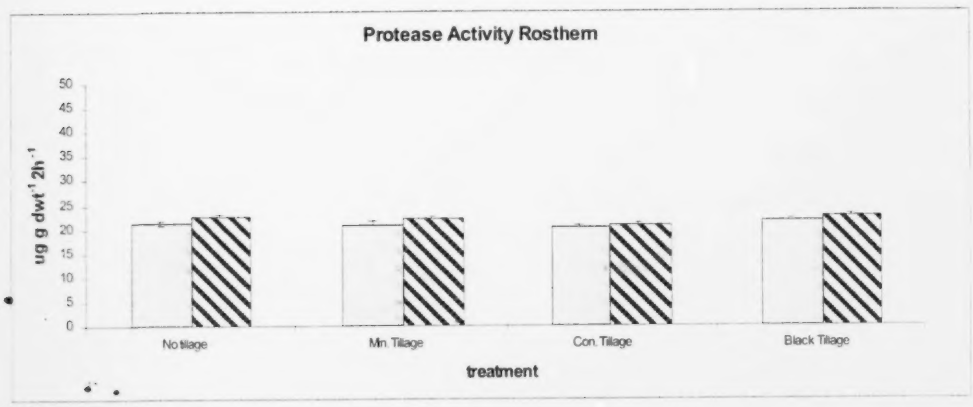


Figure 12: Net N mineralization from Tisdale long-term no-tillage plots receiving different intensities of a one-time intermittent tillage operation. May soil samples were collected immediately after the plots were tilled; July samples were collected two months after the tillage operations and October samples collected four months after the tillage operations. Different letters indicate statistical differences ($lsd_{0.10}$).

Potential denitrification measurements were highly variable and showed no patterns for any of the sampling times or sites relative to the application of the tillage treatments (data not shown). Denitrification tends to be microsite specific, occurring in "hot spots" in the soil where C and N substrates accumulate and moisture conditions limit O_2 supply. Measurements on mixed, composite, soil samples in this study did not reveal any inherent differences in denitrifying microbial populations caused by the implementation of tillage treatments.

Protease and urease activities provide additional measures of a soil's functional ability to supply N. Protease catalyses the release of inorganic N from proteins in organic matter. Almost all N in organic materials is contained in proteins. Urease catalyses the release of NH_4^+ from urea molecules. Its activity is further along the decomposition pathway, after inorganic molecules are released from organic matter. It also releases NH_4^+ from applied urea fertilizers. None of the tillage treatments impacted protease activity at any of the sites (Fig. 13). There was a difference between the May and October samples at Central Butte and Tisdale, in that activities were higher later in the season. This reflects the input of organic matter from crop harvest stimulating the microbial populations responsible for organic matter breakdown. Similarly, urease activity was not affected by tillage treatment at Central Butte nor Rosthern (Fig. 14). Urease activity was highly variable at the Tisdale site, and tended to be higher in the no-till and minimum tillage treatments at the May sampling. The higher urease activities may be responsible, in part, for the higher nitrification rates (Fig. 9) observed in these two treatments, as well as the higher overall N mineralization (Fig. 12). However, by October urease activity was the same across all tillage treatments, indicating that the sustained differences in N mineralization at this site were not due exclusively to urease function.





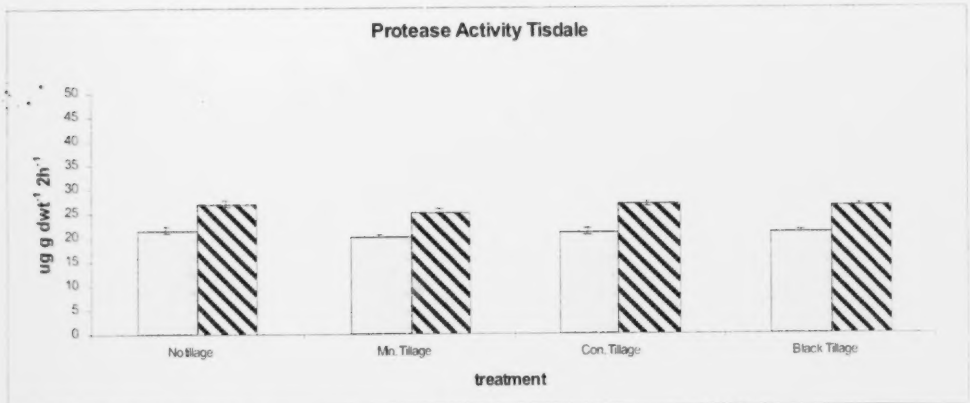


Figure 13. Protease activity at three long-term no-tillage sites tilled once with different intensities of tillage. Values (standard error) are enzyme activities in soils immediately after tillage (May) and post-harvest of the same year (October). Different letters indicate statistical differences ($lsd_{0.10}$) within a sampling time.

Figure 14. Urease activity at three long-term no-tillage sites tilled once with different intensities of tillage. Values (standard error) are enzyme activities in soils immediately after tillage (May) and post-harvest of the same year (October). Different letters indicate statistical differences ($lsd_{0.10}$) within a sampling time.

Biological controls of phosphorus availability:

Like N-mineralization, P-mineralization at Central Butte was stimulated by the imposition of the black tillage treatment (Fig. 15). There was a tendency for the black tillage treatment to have higher P-mineralization throughout the season, but the differences were only statistically different ($P>0.10$) in the May sampling.

In contrast, there was no immediate change in P mineralization with the application of the tillage treatments at Rosthern (Fig. 16). Mid-season and post-harvest, the conventional and black tillage treatments tended to have lower P-mineralization compared to the no-till controls. The differences between the tillage treatments were less pronounced in the October sampled soils than they were in the July sampled soils. The lower rates of P-mineralization were seen in the lower concentrations of plant available PO_4 in the conventional and black tillage treatments in July and October (Table 2).

At Tisdale black tillage increased P-mineralization (Fig. 17) in the May sampling, whereas the conventional tillage depressed P-mineralization. As was the case with N-mineralization, the soil mixing occurring with the black tillage treatment, probably stimulated microbial populations responsible for P mineralization. The conventional tillage treatment at this site did not sufficiently mix the soil. Although there was a tendency for the black tillage treatment for enhanced P-mineralization later in the season (July and October) the differences were not statistically significant ($P>0.10$).

Phosphatase plays a key role in the mineralization of PO_4 from organic matter. Changes in phosphatase activity do not explain differences in P-mineralization due to the imposition of tillage treatments at any of the sites (Fig. 18). Phosphatase activity differed according to the sampling time but was not affected by tillage treatment.

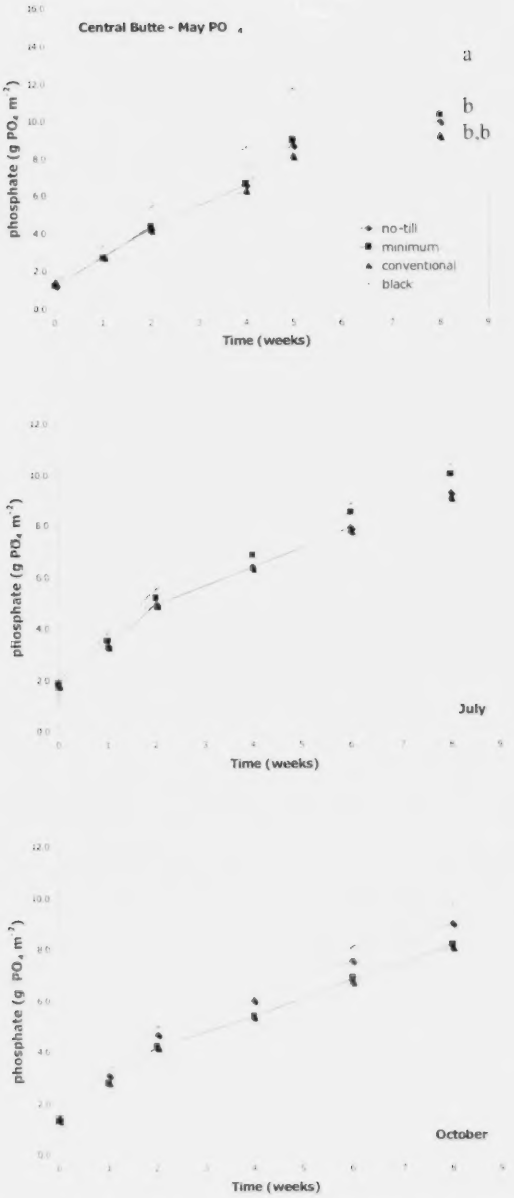


Figure 15. Cumulative phosphate production (P-mineralization) from Central Butte long-term no-tillage plots receiving different intensities of a one-time intermittent tillage operation. May soil samples were collected immediately after the plots were tilled; July samples were collected two months after the tillage operations and October samples were collected four months after the tillage operations. Different letters indicate statistical differences ($lsd_{0.10}$).

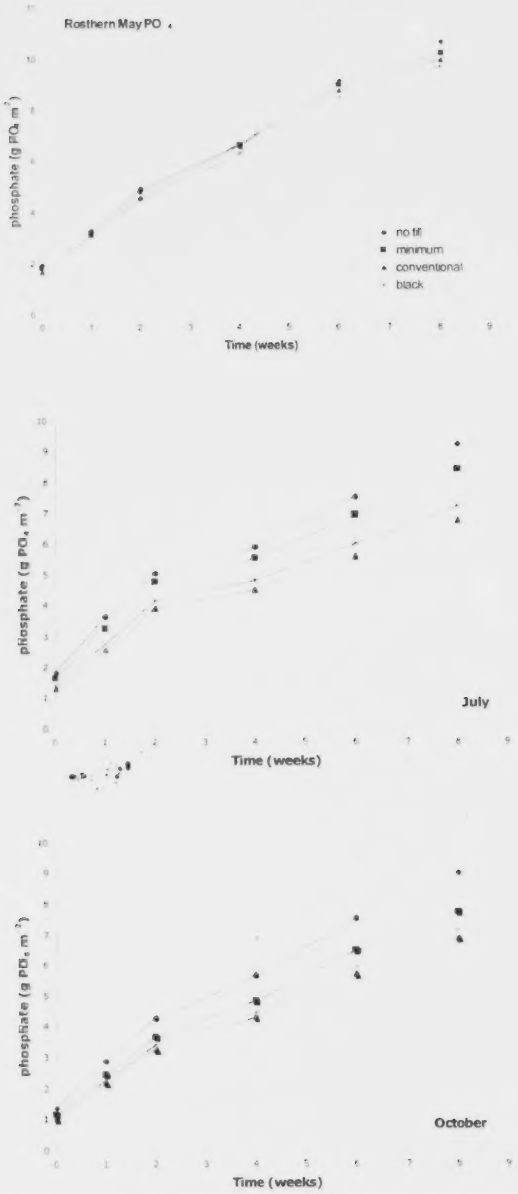


Figure 16. Cumulative phosphate production (P-mineralization) from Rosthern long-term no-tillage plots receiving different intensities of a one-time intermittent tillage operation. May soil samples were collected immediately after the plots were tilled; July samples were collected two months after the tillage operations and October samples were collected four months after the tillage operations. Different letters indicate statistical differences ($lsd_{0.10}$).

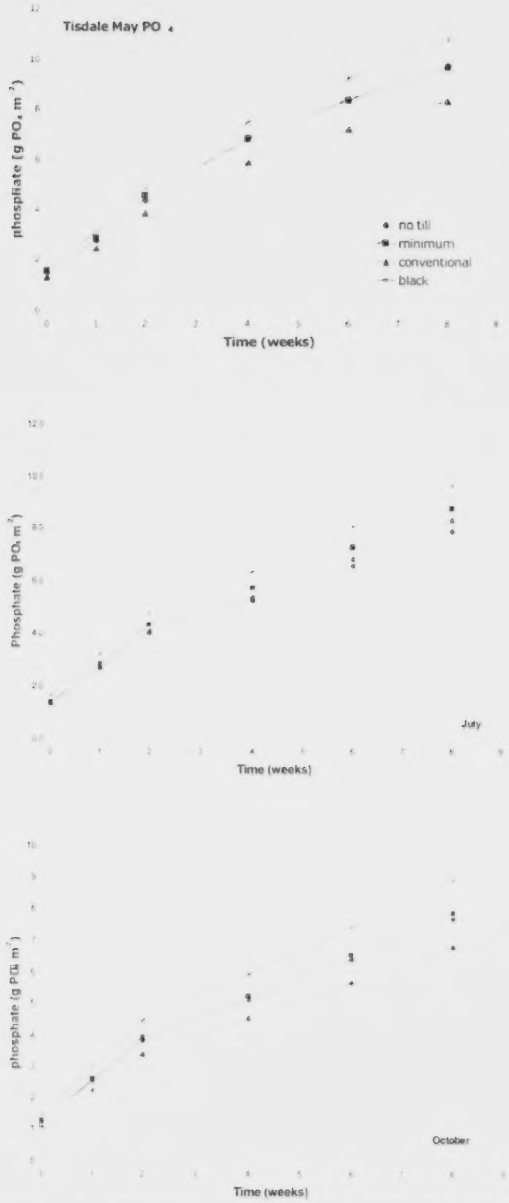


Figure 18. Cumulative phosphate production (P-mineralization) from Tisdale long-term no-tillage plots receiving different intensities of a one-time intermittent tillage operation. May soil samples were collected immediately after the plots were tilled; July samples were collected two months after the tillage operations and October samples were collected four months after the tillage operations. Different letters indicate statistical differences ($lsd_{0.10}$).

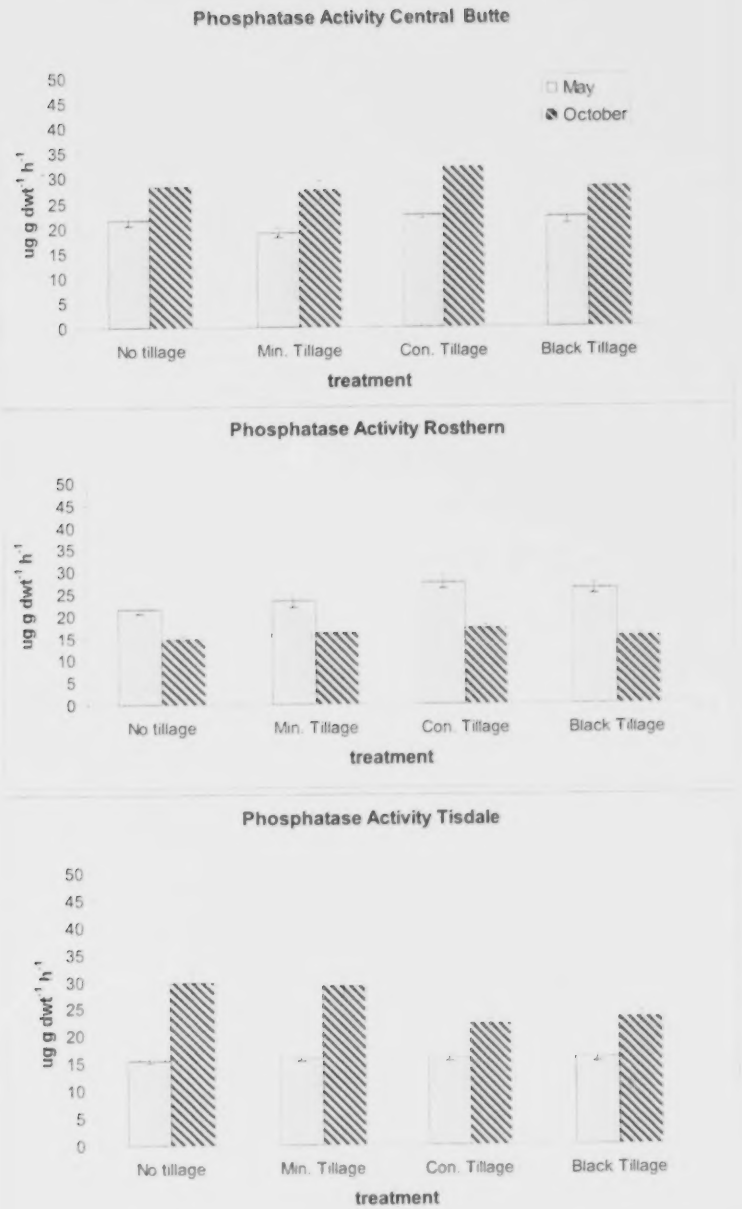


Figure 19. Phosphatase activity at three long-term no-tillage sites that were tilled once with different intensities of tillage. Values are enzyme activities (standard error) in soils immediately after tillage (May) and post-harvest of the same year (October) Different letters indicate statistical differences ($lsd_{0.10}$) within a sampling time.

Conclusions and Recommendations:

Tillage of the long-term no-till sites had very little long-term affect on indices of soil biological activity. The Central Butte site in the Brown soil zone was particularly unresponsive to the tillage treatments. Only the most intensive black tillage treatment had an early effect of increasing CO_2 emissions, nitrification and mineralization rates that were also evident in microbial biomass measurements. By the mid-season July sampling, all of the soil quality indices had stabilized back to the level observed in the no-till control plots. Tillage at the Central Butte site also did not affect grain and straw yields (Baan 2007). There appears to be very little risk of soil quality decline involved in periodically tilling this site. None-the-less, the least invasive tillage to accomplish the objective is recommended. Although the black tillage treatment did not negatively impact yields, the enhanced microbial activity in terms of CO_2 production and N and P mineralization could degrade the soil. This site has very low organic matter contents making it particularly susceptible to degradation.

The Rosthern site represented an intermediate site in terms of its organic matter content and response to the tillage treatments. In general, the black tillage treatment stimulated the mineralization of inorganic N but not P, early after the implementation of the tillage. This improved N-supply in the black tillage was related to higher grain yields at this site (Baan, 2007). The risk of depleting the organic matter supply by periodically tilling the no-till soils is less at this site than at the Central Butte site, because of the inherently higher organic matter contents.

The Tisdale site had a more complex response to the tillage treatments. This site had very high soil organic matter content (4.5% organic C) and also exhibited the most extreme nutrient stratification in the top 10 cm, compared to the other sites (Baan, 2007). In general, all of the tillage treatments depressed the soil microbiological measurements compared to the no-till controls and the degree of depression was associated with the intensity of the tillage. Several of the indices measured decreased from no-till to minimum till to conventional till, but then increased with the black tillage treatment back to near the levels of the no-till treatment. P-mineralization and microbial biomass C both exhibited this pattern. It appears that the black tillage treatment was the only tillage treatment that sufficiently mixed the soil to a degree that an advantage in terms of

nutrient availability and/or microbial stimulation was achieved. In the related study performed by Baan (2007) soil NO_3^- levels in the top 5 cm of the black tillage treatment (were approximately one-half of the levels in the no-till treatment because of soil mixing. Grain yields followed the same pattern in that yields were reduced in the minimum and conventionally tilled treatments, and rose to near the same level in the black tillage treatment (Baan, 2007). Considering the very high inherent organic matter levels in this soil, there should be little risk of depleting organic matter due to the intensive tillage treatment. In this Tisdale soil the most intensive tillage treatment appears to be the least detrimental to crop productivity.

One draw back of both this and the related Baan (2007) study is that perennial weeds and disease incidence was not monitored to determine if the intermittent tillage was successful in controlling them. While the intermittent tillage was generally not detrimental to the long-term no-till field productivity, or soil quality, it should only be implemented with a specific management objective (like weed or disease control) in mind.

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